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Total Synthesis of Syringolin A and B

Michael C. Pirrung,* Goutam Biswas, and Tannya R. Ibarra-Rivera

Department of Chemistry, University of California, Riverside, California 92521

michael.pirrung@ucr.edu

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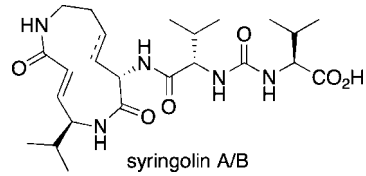
ABSTRACT



syringolin A/B

Total syntheses of two recently discovered proteasome inhibitors, syringolin A and B, are reported. The key to our approach was creation of the α,β-unsaturated 12-membered lactam via intramolecular Horner−Wadsworth−Emmons reaction. Such reactions have been broadly used to prepared macrolactones, but this work presents a rarer example of its application to macrolactams. The final steps involved attachment of the bis(valinyl)urea side chain using peptide coupling procedures, including a method based on the unprotected valine *N*-carboxy anhydride. The additional alkene of syringolin A was created through cross-metathesis.

Inactivation of the proteasome has recently been identified as the mode of action of a variety of anticancer agents, both natural products and synthetic compounds. These inhibitors exploit the mechanism used by the proteasome, nucleophilic catalysis by the N-terminal threonine,[1] to trigger a reaction with an electrophilic functional group in the inhibitor. The types of electrophiles that are known to react with the catalytic Thr1 of the proteasome include boronic acids, β-lactones, epoxyketones,[2] and cyclic thionocarbonates.[3]

In 2008, two macrolactams, syringolin A and B, were shown to affect plant−pathogen interactions[4] and to act as virulence factors via inhibition of the proteasome.[5] They are members of a bioactive natural product family that has been known for many years, includes the glidobactins/cepafungins, and is now called the syrbactins. Crystallography established that both syringolin A and B form a covalent adduct with the proteasome via conjugate addition of the N-terminal threonine hydroxyl group to their α,β-unsaturated lactams. Given the known properties of proteasome inhibitors, the action of syringolin A in cancer cells was investigated. Treatment of a human neuroblastoma cell line with 25 μM syringolin A causes a dose-dependent decrease in proteasome activity and a time-dependent accumulation of ubiquitinated proteins via irreversible inhibition of the proteasome.

These biological properties stimulated our interest in the synthesis of the syringolins. Structures based on the syrbactins would be valuable to access by total synthesis in order to (1) develop novel anticancer therapeutics, (2) better understand plant host−pathogen interactions, and (3) elucidate fundamental questions concerning the structure and function of the proteasome in diverse eukaryotes, including higher plants and even mycobacteria.[3]

Creation of the macrolactam poses the major challenge for the synthesis of the syringolins. The Kaiser group recently completed syntheses of both syringolin A and B.[6] Syringolin A was dissected into the bis(valinyl)urea side chain and the 12-membered macrolactam core. The

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core was prepared with a protected diol as a synthon for the *trans*-α,*β*-unsaturated amide, and the formation of the macrolactam was achieved by ring-closing metathesis in 49% yield. After side chain attachment, the unsaturated amide was revealed in the penultimate step. This synthesis entails 16 linear steps (not including preparation of the side chain) and proceeds in an excellent 9% overall yield. The syringolin B synthesis used lysine and valine as starting materials, assembled a polypeptide using standard coupling methods, and then used a peptide coupling agent at high dilution to close the macrolactam, which proceeded in 30% yield. Glidobactin A, a natural product with a core ring closely related to the syringolins, has also been synthesized.[7] Here, macrolactamization via a pentafluorophenyl active ester gave the 12-membered ring in only 20% yield.

Our aim was a modular and general synthetic approach to the syringolins with the potential for straightforward preparation of structural variants simply by the substitution of structurally variant modules (diversity-oriented synthesis).[8] The α,*β*-unsaturated amide of the syringolins suggested an intramolecular Horner−Wadsworth−Emmons condensation for the preparation of the 12-membered ring. Such reactions have found utility in the high-yield synthesis of macrolactones[9] but have been applied to only a few macrolactams.[10]

The total synthesis of syringolin B was a proving ground for the macrocyclization. The C_2-symmetric bis(valinyl) urea side chain was prepared from two commercial compounds: L-valine-derived isocyanate **1** and L-valine *tert*-butyl ester hydrochloride, using a route developed by Clerc et al.[6] The monoacid was activated as the nicely crystalline NHS active ester **2**. Boc-L-Lysine was acylated with the NHS active ester of diethylphosphonoacetic acid (**3**) using a protocol developed for other acids,[11] and then a conventional peptide coupling with L-valinol was executed. The two-step yield of **4** is 75%. Its Dess−Martin oxidation sets up the key cylization reaction. The very mild Horner−Wadsworth−Emmons protocol developed by Helquist for the formation of acrylamides with high selectivity for (*E*) stereochemistry was uniquely successful.[12] This contrasted with the outcome using several other common methods. Helquist's method was applied using 2 equiv of Zn^{2+}, 1 equiv of TMEDA, and 4 equiv of Et_3N for 15 h at 5 mM concentration; the cyclization product **5** was obtained in 75% yield. No stereoisomers (NMR and HPLC data) or dimers (MS data) were detected in this product. The removal of the Boc group was performed with HBr in acetic acid to give the amine salt in 100% yield, and the free base was obtained by treatment with the ion-exchange resin MP-carbonate in methanol. Coupling of the amine with **2**, a route similar to that used by Clerc et al. for syringolin A,[6] was disappointing, giving yields of **7** of ca. 50%.

More reactive and sterically smaller acylating agents for the macrolactam amine were therefore sought, and amino acid *N*-carboxy anhydrides were considered. While such reagents were popularized for solid-phase peptide synthesis as the urethane derivatives,[13] several examples are known of peptide bond formation using free amino acid *N*-carboxy anhydrides, which should be among the most sterically undemanding of amino acid acylating reagents. Although they can undergo ring-opening polymerization, the use of organic solvents and low temperatures has enabled them to be used for monoacylation reactions.[14] The appeal of this strategy is enhanced by the commercial availability of many amino acid *N*-carboxy anhydrides, as well as excellent procedures to prepare them from Boc-amino acids.[15] In the event, the free base derived from **5** was treated with the commercially available valine *N*-carboxy anhydride **6** in DMF/dichloromethane for 4 h at −78 °C and then 2 h at rt. Without purification, the acylation mixture was subjected to urea formation with **1**, and the ester **7** was obtained in 55% yield. As this route obviates the advanced preparation of reagents like **2**, it considerably streamlines side chain preparation. It also makes the synthesis more modular, enabling the simple preparation of side chain variants by subsitution of the isocyanate and *N*-carboxy anhydride components. Finally, it may be considered biomimetic, as recent studies on the biosynthesis of the syringolins have invoked *N*-carboxy anhydride **6** as one possible intermediate in the formation of this unusual amino acid urea.[16] The saponification of **7** gave syringolin B, which exhibited spectroscopic properties (^{1}H NMR, ^{13}C NMR) matching those reported for the natural product.

With the phosphonate macrocyclization proven in the syringolin B synthesis, we moved on to syringolin A (Scheme 2). We required a method to prepare 3,4-dehydrolysine intermediates, and the strategy that emerged to address this need made for an even more modular synthesis. Modules used for the macrolactam core include

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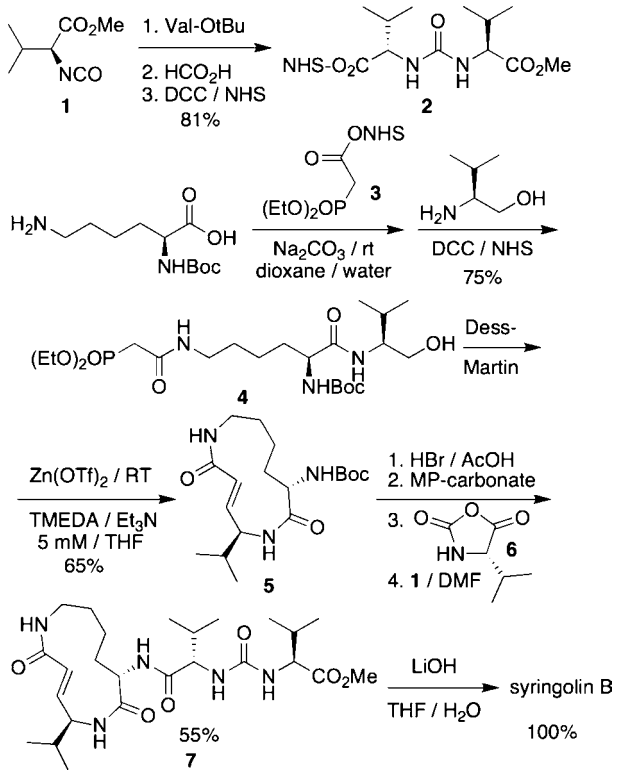
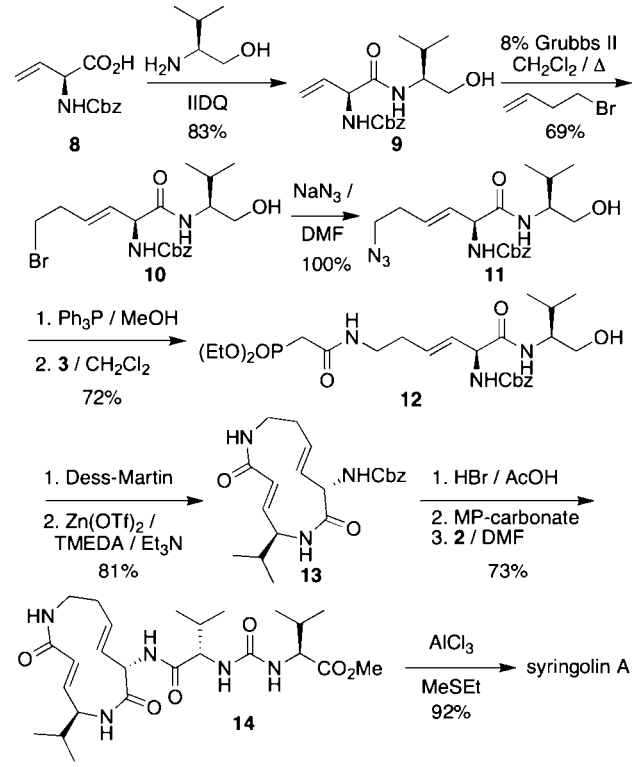
Scheme 1. Total Synthesis of Syringolin B



Scheme 2. Total Synthesis of Syringolin A



valinol and **3** from the first synthesis, vinyl glycine derivative **8**, and 1-bromo-3-butene. Compound **8** is available in three steps from commercial *Z*-Met-OMe.[17] Peptide coupling of vinyl glycine derivatives is known using conventional mixed anhydride protocols as well as the more exotic reagent 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquin-oline (IIDQ).[18] These studies prepared vinyl glycine amides for use in following metathesis reactions, as performed here. The coupling of L-valinol with **8** gives **9** efficiently, though slowly. This product can be crystallized in stereochemically homogeneous form. This coupling is significantly enhanced by microwave irradiation (80 °C internal temperature, CEM Discover), wherein it can be completed in 10 min. We take advantage of the commercial availability of 1-bromo-3-butene to use a 10-fold excess in cross-metathesis[19] with **9** using the Grubbs second-generation catalyst.[20] This gives a product **10** that has exclusively the (*E*) stereochemistry. Nucleophilic substitution of **10** with sodium azide introduces the last nitrogen. Staudinger reduction of the azide and then coupling with the phosphonoacetic acid active ester **3** give **12** (72% for two steps). The oxidation and cyclization of this material (2 equiv Zn^{2+}, 1 equiv TMEDA, 8 equiv TEA, rt, 2 mM, 12 h) is nearly as successful as in the first synthesis (55%). When the aldehyde is added to the reaction mixture over 8 h, the yield increases to an outstanding 81%. While the presence of two alkenes in the 12-membered ring is expected to increase the strain relative to syringolin B (calculated by MMFF to be 13 kcal/mol), this does not appear to be a major impediment to the cyclization reaction. Again, no stereoisomers are detected in product **13**. This synthesis was completed by removal of the Cbz group with HBr in acetic acid and acylation with **2**. This process was enhanced by neutralizing the amine salt with the ion-exchange resin MP-carbonate in DMF and performing the acylation in the same pot. The known ester **14** is obtained in 73% yield. The final deprotection used a high-yielding procedure reported by Clerc et al.[6] Synthetic syringolin A gave spectroscopic and physicochemical properties matching those reported for the natural product and was identical by comparison to an authentic sample (^{1}H NMR, ^{13}C NMR, HPLC).

These syntheses proceed in 7 steps and 27% overall yield and 10 steps and 22% overall yield. The number of functional group interconversions and redox steps was

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minimized by the use of commercial and amino acid derived building blocks. These syntheses efficiently construct the large lactam rings via the Horner−Wadsworth−Emmons reaction and in particular use mild reaction conditions developed by Helquist that were uniquely effective in these cyclizations. Valine *N*-carboxy anhydride proved a suitably active reagent to efficiently acylate the somewhat hindered and electronically deactivated syringolin B macrolactam amine. These syntheses are quite modular and should permit the preparation of many structural variants that could improve on the biological properties of the parent natural products.

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Supporting Information Available: NMR spectra for new compounds and other experimentals and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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